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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (b)(2).

Docket Number	10461/003001	Type a plus sign (+) inside this box →	+

INVENTOR(s)/APPLICANT(s)

LAST NAME	FIRST NAME	MIDDLE NAME/INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
Naviaux	Robert	K.	San Diego, CA

TITLE OF THE INVENTION (280 characters max)

THE USE OF PN401 IN THE TREATMENT OF MITOCHONDRIAL DISEASE

"Express Mail" mailing label number EL253771766US
Date of Deposit February 23, 1999
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M. E. Kavanaugh
M. E. Kavanaugh

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ENCLOSED APPLICATION PARTS *(check all that apply)*

[X]	Specification	*Number of Pages*	20 pp.	[X]	Small Entity Statement
[ ]	Drawing(s)	*Number of Sheets*		[ ]	Other (specify)

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

[X]	A check or money order is enclosed to cover the filing fees	FILING FEE AMOUNT ($)	$75.00
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No.

[ ] Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE Lisa A. Haile Date 2/23/99

TYPED or PRINTED NAME Lisa A. Haile, Ph.D. REGISTRATION NO. (if appropriate) 38,347

[ ] Additional inventors are being named on separately numbered sheets attached hereto

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:
Attorney's Docket No.:
Title:
UCSD Case No.:

VERIFIED STATEMENT UNDER 37 CFR §1.9(f) AND §1.27(d) CLAIMING STATUS AS A SMALL ENTITY -- NONPROFIT ORGANIZATION

I, Dr. Alan Paau, Director of the Technology Transfer Office of the University of California, San Diego, hereby declare that:

1. I am empowered to act on behalf of THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, a corporation duly organized under and pursuant to the laws of CALIFORNIA having its principal place of business at 300 Lakeside Drive, 22nd Floor, Oakland California 94612-3550, in the matter of this VERIFIED STATEMENT.

2. THE REGENTS OF THE UNIVERSITY OF CALIFORNIA is a University of higher education of the State of California and a nonprofit organization.

3. The rights under contract or law in the invention contained within the above identified patent application are obligated to be assigned to and remain with THE REGENTS OF THE UNIVERSITY OF CALIFORNIA. THE REGENTS OF THE UNIVERSITY OF CALIFORNIA has not assigned, granted, conveyed or licensed, and is under no obligation under contract or law to assign, grant, convey or license any rights in the invention nor in the application to any person who could not be classified as an independent inventor if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR §1.9(d) or a non-profit organization under 37 CFR §1.9(e).

4. THE REGENTS OF THE UNIVERSITY OF CALIFORNIA qualifies as a nonprofit organization as defined in 37 CFR §1.9(e) for paying reduced fees under section41 subsections (a) and (b) of Title 35, United States Code, with regard to said application.

5. I acknowledge the duty under 37 CFR §1.28(b) to file, in this application or any patent issuing thereon, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of any issuance fee or any maintenance fee due after that date on which status as a small entity is no longer appropriate.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent(s) issuing thereon.

2/23/99
Date

Alan Paau
Signature of Alan Paau, M.B.A., Ph.D.
Director, Technology Transfer Office
University of California San Diego
9500 Gilman Drive, MC 0910
La Jolla, CA 92093-0910
Representative of THE REGENTS OF THE UNIVERSITY OF CALIFORNIA

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PROVISIONAL APPLICATION

UNDER 37 CFR 1.53(b)(2)

TITLE: THE USE OF PN401 IN THE TREATMENT OF MITOCHONDRIAL DISEASE

APPLICANT: ROBERT K. NAVIAUX

"EXPRESS MAIL" Mailing Label Number EL253771766US
Date of Deposit February 23, 1999
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M.E. Augustine

M.E. Augustine

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Backgr und

Mitochondrial diseases occur as inherited, sporadic, and acquired forms. Inherited forms of mitoch ndrial disease have a high mortality and morbidity. The most severe forms, such as Leigh syndrome (subacute necrotizing encephalomyelopathy) have a mortality of up to 50% per year after diagnosis. In a UCSD study of 40 patients with mitochondrial disease treated with sodium dichloroacetate (DCA), 10 patients had severe disease progression (25%) and 6 patients died (15%) within 8 weeks of entering the study (unpublished results). Multifactorial forms of mitochondrial disease include much more common disorders such as Parkinson disease, Alzheimer disease, and even certain forms of diabetes, heart disease, and stroke. Indeed the process of aging itself has been linked to progressive declines in mitochondrial function (ref).

Mitochondrial diseases are defined as disorders of mitochondrial metabolism that arise from a genetic defect in nuclear or mitochondrial DNA. These may be maternally inherited, inherited as conventional Mendelian disorders, or acquired as new somatic mutations. The disorders may be manifested at any genetic level, from DNA and RNA, to protein. They may affect mitochondrial DNA replication, transcription, the transport of macromolecules into or out of mitochondria, or the function of macromolecules at their site of action within mitochondria. Historically, discussions of pathogenesis in mitochondrial disease have focused on the degradative (oxidative) functions of mitochondria. However, a number of the symptoms of mitochondrial disease may be related to essential biosynthetic (non-degradative) functions of the organelles that are often overlooked. One biosynthetic function of mitochondria is the synthesis of uridine. This patent claim is based on my clinical findings that uridine and PN401 (triacetyluridine) are effective at correcting or improving many of the symptoms of mitochondrial disease.

The epidemiology of the inherited forms of mitochondrial disease is largely unknown. It has been estimated that between 1 in 4000 and 1 in 1000 live births in the U.S. will be diagnosed with a mitochondrial disease before the age of 10 years. This is roughly comparable to the incidence of childhood cancer. Degenerative disorders of aging in which mitochondria play a role are, of course, much more common, affecting as many as 20-85 million Americans (see Fig. 1).

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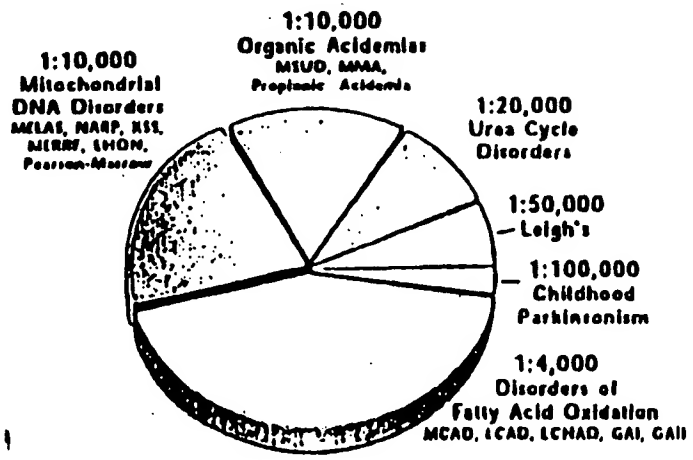
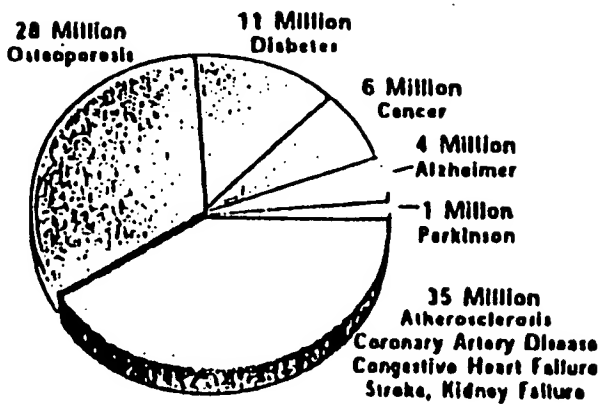
The Epidemiology of Mitochondrial Disease

Childhood Mitochondrial Disease



Adult Diseases with Mitochondrial Dysfunction



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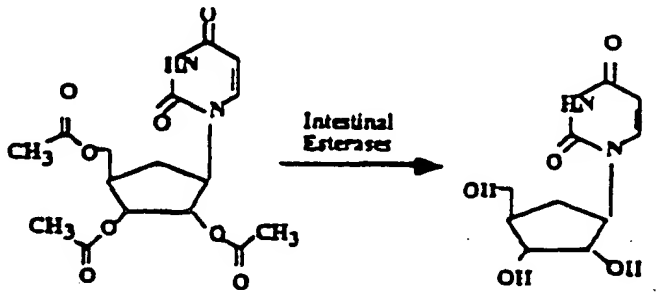
Concept

PN401 is tri-O-2',3',5'-acetyluridine, also called triacetyluridine. PN401 is simply a prodrug of uridine that is rapidly converted to uridine by non-specific esterases in the gastrointestinal epithelium after oral delivery (*Kelson DP, et al. J Clin Onc 15:1511-1517, 1997*). PN401 has 5-10 times the bioavailability of unmodified uridine. This permits the achievement of higher therapeutic blood levels of uridine, with substantially less drug, than those achieved by giving unmodified uridine by mouth. The only detectable metabolites of PN401 are uridine, uracil, and the catabolites of these molecules. No partially acetylated forms of uridine are detectable in the plasma after oral administration of triacetyluridine, PN401.



PN401
MW = 370

Uridine
MW = 244

Patients with a variety of different mitochondrial disorders may be functionally deficient in uridine because the rate-limiting step in *de novo* pyrimidine synthesis (Dihydroorotate CoQ Oxidoreductase, EC 1.3.99.11) is located on the inner membrane of mitochondria and coupled to the electron transport chain. Cells with mitochondrial dysfunction in culture are known to be dependent on exogenous uridine for growth and survival because of a functional deficiency in the activity of DHO-QO.

Mitochondrial Disorders

The following is a list of some of the known mitochondrial disorders:

1. MELAS (Mitochondrial encephalomyopathy with lactic acidemia and stroke-like episodes)
2. MERRF (Myoclonus, epilepsy, and myopathy with ragged red fibers)
3. NARP/MILS (Neurogenic muscular weakness, ataxia, retinitis pigment sa/Maternally inherited

Leigh syndrome)
4. LHON (Lebers hereditary optic neuropathy) "Mitochondrial blindness"
5. KSS (Kearns-Sayre Syndrome)
6. PMPS (Pearson Marrow-Pancreas Syndrome)
7. CPEO (Chronic progressive external ophthalmoplegia)
8. Leigh syndrome
9. Alpers syndrome
10. Multiple mtDNA deletion syndrome
11. MtDNA depletion syndrome
12. Complex I deficiency
13. Complex II (SDH) deficiency
14. Complex III deficiency
15. Cytochrome c oxidase (COX, Complex IV) deficiency
16. Complex V deficiency
17. Adenine Nucleotide Translocator (ANT) deficiency
18. Pyruvate dehydrogenase (PDH) deficiency
19. Ethylmalonic aciduria with lactic acidemia
20. 3-Methyl glutaconic aciduria with lactic acidemia
21. Refractory epilepsy with declines during infection--a subpopulation of these patients
22. Asperger syndrome with declines during infection--a subpopulation of these patients
23. Autism with declines during infection--a subpopulation of these patients
24. Attention deficit hyperactivity disorder (ADHD) with declines during infection--a subpopulation of these patients
25. Cerebral palsy with declines during infection--a subpopulation of these patients
26. Dyslexia with declines during infection--a subpopulation of these patients
27. Maternally inherited thrombocytopenia and leukemia syndrome
28. MNGIE (Mitochondrial myopathy, peripheral and autonomic neuropathy, gastrointestinal dysfunction, and epilepsy)
29. MARIAHS syndrome (Mitochondrial ataxia, recurrent infections, aphasia, hypouricemia/hypomyelination, seizures, and dicarboxylic aciduria)
30. ND6 dystonia
31. Cyclic vomiting syndrome with declines during infection--a subpopulation of these patients
32. 3-Hydroxy isobutyric aciduria with lactic acidemia
33. Diabetes mellitus with lactic acidemia
34. Uridine responsive neurologic syndrome (URNS)--this group of patients is being studied by Dr. Alice Yu, who currently has an approved human subjects protocol focused on this clinical phenotype.
35. Familial Bilateral Striatal Necrosis (FBSN)
36. Aminoglycoside-associated deafness (1555 mutation in mtDNA)
37. Dilated cardiomyopathy--mtDNA mutations occur in a subpopulation of these patients
38. Splenic Lymphoma--mtDNA mutations occur in a subpopulation of these patients
39. Sideroblastic Anemia--mtDNA mutations occur in a subpopulation of these patients
40. Wolfram syndrome
41. Multiple mitochondrial DNA deletion syndromes
42. Renal Tubular Acidosis/Diabetes/Ataxia syndromes

<u>Other conditions with known or suspected mitochondrial dysfunction which may benefit from PN401</u>

1. Post-traumatic head injury and cerebral edema
2. Stroke--Primary prevention or Prevention of reperfusion injury
3. Alzheimer dementia
4. Lewy body dementia

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5. Parkinson disease
6. Hepatorenal Syndrome
7. Acute Liver Failure--NASH (non-alcoholic steat hepatitis) and thers
8. Diabetes mellitus (Type II >> Type I)
9. Anti-metastasis/prodifferentiation therapy of cancer
10. Idiopathic congestive heart failure
11. Atrial fibrillation--nonvalvular
12. Wolff-Parkinson-White syndrome
13. Idiopathic heart block
14. Prevention of reperfusion injury in acute myocardial infarctions
15. Familial Migraines
16. Irritable bowel syndrome
17. Secondary prevention of non-Q wave myocardial infarctions
18. Premenstrual Syndrome
19. Prevention of renal failure in hepatorenal syndrome
20. Anti-phospholid Antibody Syndrome
21. Eclampsia/Pre-eclampsia
22. Oopause infertility
23. Ischemic heart disease/Angina
24. Shy-Drager and unclassified Dysautonomia syndromes

Biochemical Rationale of Uridine Therapy of Mitochondrial Disease

The synthesis, transport, and efficient interconversions of pyrimidines are essential for normal cell and organ function. All cellular pyrimidines are ultimately derived from the synthesis of uridine, which in turn, must be synthesized from orotic acid by condensation with phosphoribosyl pyrophosphate (PRPP) and decarboxylation. The fourth and rate limiting step in the synthesis of pyrimidines is catalyzed by dihydroorotate CoQ10 oxidoreductase (DHO-QO, EC 1.3.99.11; also called dihydroorotate dehydrogenase (DHOD). This enzyme is located exclusively on the inner mitochondrial membrane and is coupled to the electron transport chain via coenzyme Q10 (ubiquinone). See Fig. 2. Defects in oxidative phosphorylation that produce a redox imbalance block the *de novo* synthesis of pyrimidines by interfering with the transfer of electrons from DHO-QO to the oxidized form coenzyme Q10. Cells with redox disturbances then become completely dependent on an exogenous supply of pyrimidines in the form of uridine. Because children and adults with mitochondrial disease often have defects in oxidative phosphorylation, they may not be able to make enough uridine to meet their needs. Uridine then becomes an essential nutrient in the diet. Uridine, but not orotic acid, readily passes the blood brain barrier via a pyrimidine transporter, and is rapidly taken into cells and converted to UMP, UDP, and UTP (*Spector R. Uridine transport and metabolism in the central nervous system. J Neurochem 1985;45:1411-1418*).

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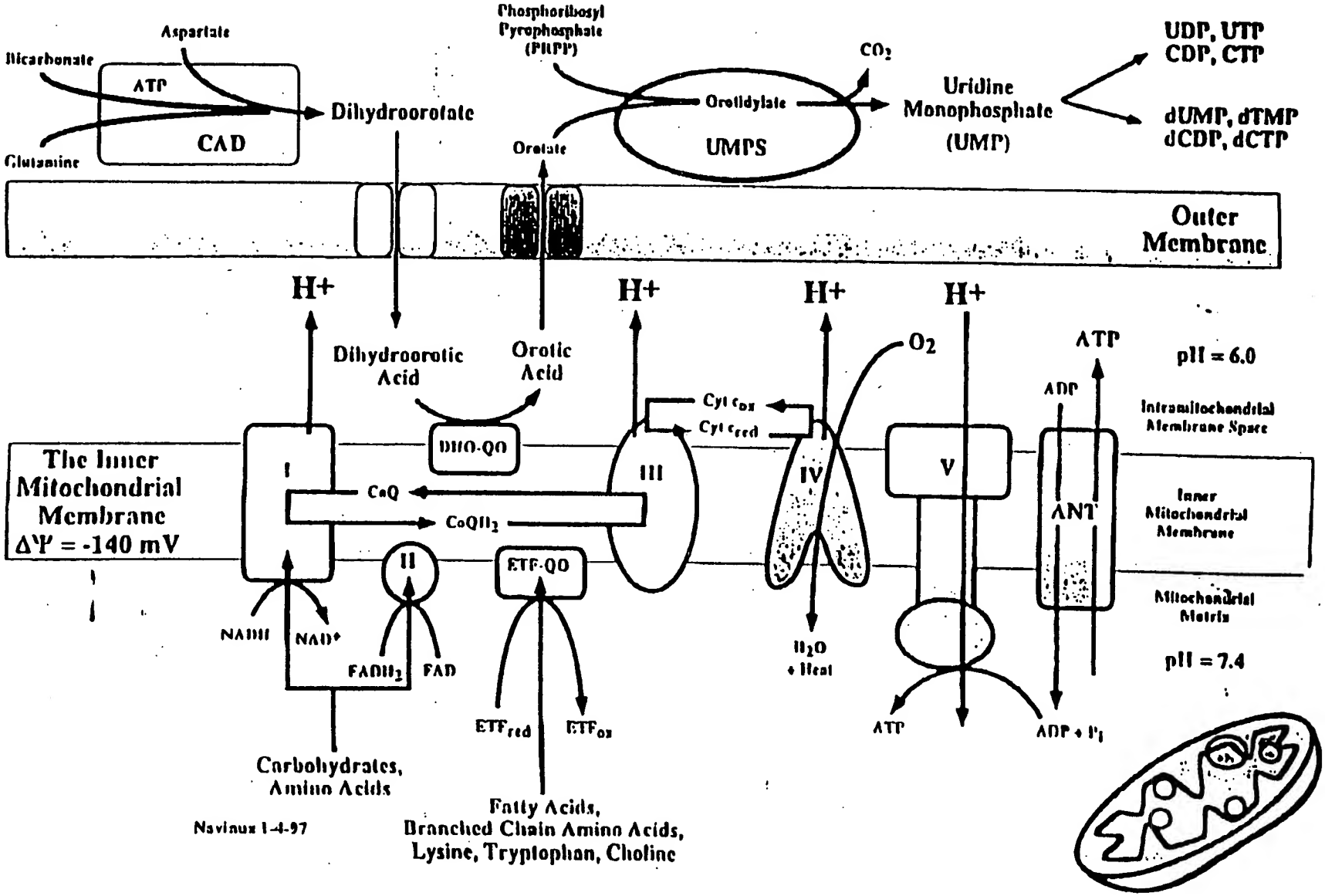
De novo Pyrimidine Synthesis is Coupled to The Mitochondrial Electron Transport Chain



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De nov synthesis of uridine (as UMP) requires 5 mol of ATP, while reuptake of uridine and phosphorylati n to UMP requires just 1 mol of ATP. The brain, heart, and skeletal muscle actively utilize this salvage pathway for pyrimidine and purine needs, importing and concentrating pre-formed nucleosides made in the liver and transported in the blood. This occurs despite the demonstrable presence of most of the enzymes required for *de novo* synthesis (*Santos JN. J Neurochem 1968;15:367-376*) and results in an 80% savings of ATP required for uridine synthesis. In addition, supplemental uridine is predicted to spare phosphoribosyl pyrophosphate (PRPP) which is required in the *de novo* synthesis of both purines and pyrimidines.

Indirect Effects on Oxidative Phosphorylation

Cardiolipin is a unique diphospholipid that is essential for normal oxidative phosphorylation, and found exclusively in the inner mitochondrial membrane. One of the many effects cardiolipin is to stabilize the assembly of cytochrome c oxidase (COX, complex IV). Because cardiolipin is essential for normal oxidative phosphorylation, depletion will lead to reduced ATP synthesis, and restoration of normal cardiolipin synthesis will facilitate more normal cellular bioenergetics. Activated cytidine-diphosphate (derived from uridine) is essential for all cardiolipin synthesis. In theory, if the supply of intracellular pyrimidines is limiting for cardiolipin synthesis, then normal synthesis may be restored by providing the cell with an exogenous source of pyrimidines in the form of triacetyluridine (PN401).

Metabolic Fates and Functions of Uridine

The metabolic fates of uridine range from RNA, DNA, and protein synthesis, to cofactors and activated intermediates of carbohydrate, glycoprotein, glycolipid and phospholipid metabolism (see Fig. 3). UDP-glucose, UDP-galactose, and UDP-N-galactosamine are required for accurate post-translational modification for protein trafficking, membrane ion channel, receptor and intracellular glycoprotein and glycolipid synthesis. UDP-glucose is required for glycogen synthesis and the prevention of fasting hypoglycemia. UDP-glucuronate is required for drug, steroid, and bilirubin excretion by glucuronidation. In addition, uridine triphosphate is converted to cytidine triphosphate by glutamine-dependent amination catalyzed by CTP synthetase, and dUMP is converted to thymidine monophosphate by folate-dependent methylation catalyzed by

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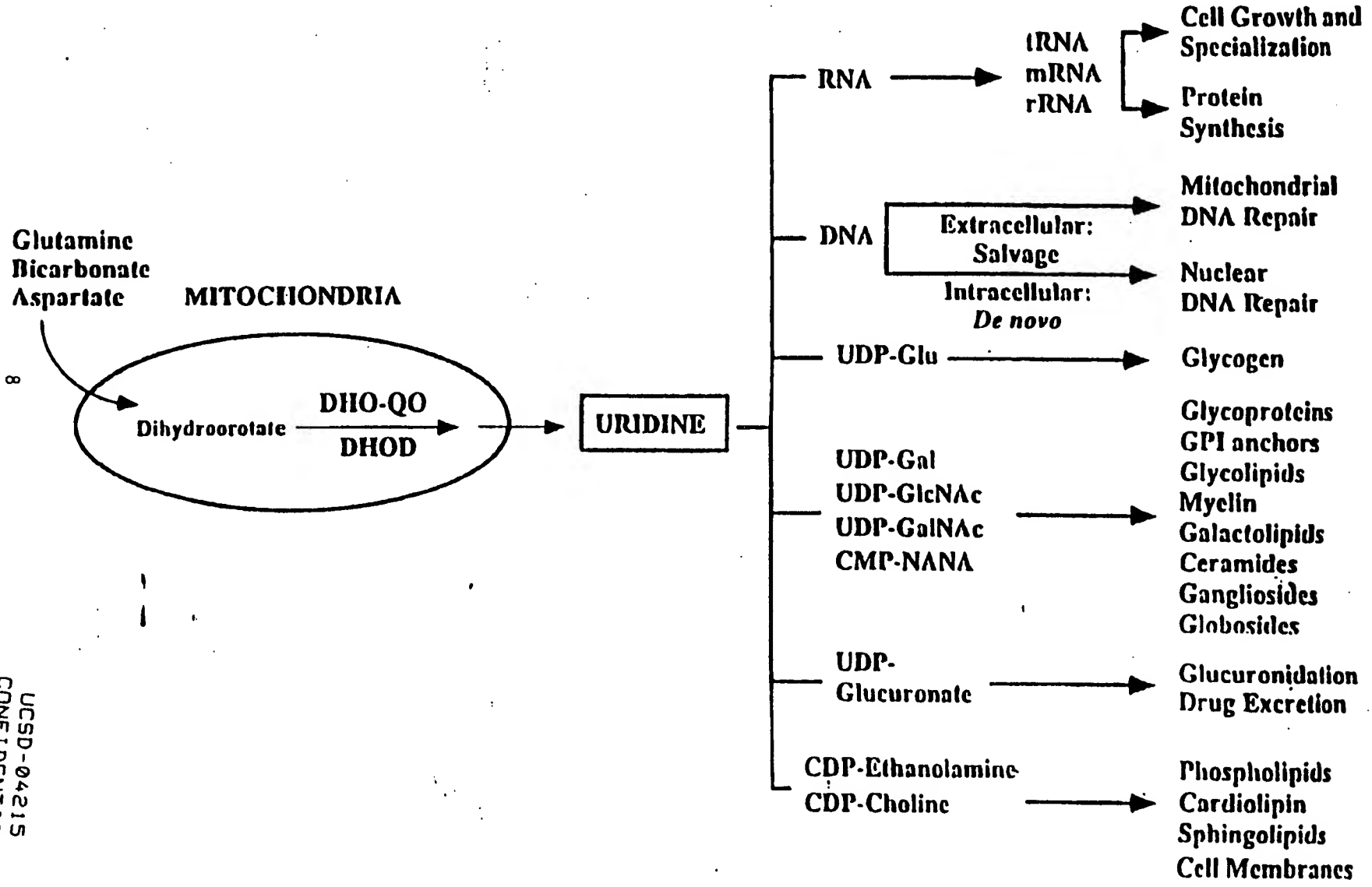
Fig. 3

Metabolic Fates and Functions of Uridine



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thymidylate synthetase. Finally, CDP-choline and CDP-ethanolamine are essential precursors for all cell membrane and myelin phospholipid synthesis. See Figure 1 for a summary of the fates and functions of uridine.

Pyrimidine Deficiency States

The only well characterized inborn error that produces a primary deficiency in uridine biosynthesis is hereditary orotic aciduria. This disorder results from a defect in the bifunctional enzyme that catalyzes the 5th and 6th steps of *de novo* pyrimidine synthesis, collectively called the UMPS reaction. The catalytic activities that are deficient are orotate phosphoribosyltransferase and orotidine decarboxylase. Enzymatic deficiency produces failure to thrive, megaloblastic anemia, orotic aciduria, minor congenital abnormalities, transient immunoglobulin deficiency, language and motor delays, and immune deficiency. Most of these abnormalities (not the structural abnormalities present in some) were reversed with 150-200 mg/kg/d (but not 100 mg/kg/d) of uridine divided TID (Webster DR *in* Scriver CR, The Metabolic and Molecular Basis of Inherited Disease, 7th ed., McGraw Hill, San Francisco, 1995).

Reproductive Effects of Longterm Uridine Usage

Patients with confirmed UMPS deficiency are alive and well in their 20's and 30's, with IQs of 66-133. Some are married and have had healthy children. Because the requirement for supplemental uridine is lifelong, the longitudinal data from these patients supports the reproductive safety of longterm (over 20 years) uridine use.

In a less well defined biochemical syndrome of PRPP synthetase deficiency, one patient reported had 15% of normal activity in fibroblasts, but normal PRPP synthetase activity in erythrocytes (Wada Y. Tohoku J Exp Med 1974;113:149-157). The patient had developmental delay, hypouricosuria, seizures, and autistic features. Defects in PRPP synthesis would be expected to produce abnormalities in purine, pyrimidine, and NAD metabolism.

A related disorder that produces a secondary deficiency in available uridine nucleotides is a uridine-nucleotide depletion disease (UNDD) in which there is superactivity of the catabolic enzyme 5' nucleotidase (PNAS 1997;94:11601-11606). These children also had seizures,

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developmental delay, hypouricemia, ataxia, and behavi ral abnormalities. Skin fibroblasts had a 5-fold increase in 5' nucleotidase activity, failed to show the normal allosteric activation by ATP, and had a 2-fold reduction in PRPP.

Appetite Stimulatory Effects of Uridine

In uridine deficiency states associated with orotic aciduria (UMPS deficiency), uridine supplementation resulted in increased appetite, increased food intake, and rapid catch up growth (Webster DR *in* Scriver CR, The Metabolic and Molecular Basis of Inherited Disease, 7th ed., McGraw Hill, San Francisco, 1995).

Anticonvulsant Effects and Neuropharmacology of Uridine

The anticonvulsant effects of uridine were discovered accidentally in the 1960's and 70's when investigators were studying RNA synthesis after seizures in experimental animals. Injection of ^{3}H-uridine itself was found to stop seizure activity induced by metrazol or penicillin (*Roberts C. Anticonvulsant effects of uridine: comparative analysis of metrazol and penicillin induced foci. Brain Research 1973;55:291-308*). Earlier studies had shown a selective effect of uridine nucleotides on neuronal membrane hyperpolarization and stabilization, but seizure models had not been explicitly tested (*Bonavita V. Electrophysiological and neurochemical studies following injection of mononucleotides and their derivatives. J Neurochem 1963;10:155-164*). Uridine competes with GABA for binding at both its high and low affinity receptors; Ki = 0.05-0.5 mM. It was an antagonist of bicuculline-induced seizures (*Guarneri P. Interaction between uridine and GABA-mediated inhibitory transmission: studies in vivo and in vitro. Epilepsia 1985; 26:666-671*). Uridine also appears to have effects on dopaminergic pathways, as it reduces amphetamine-induced dopamine release in experimental animals (*Myers C. Pharm Biochem Behav 1995;52:749-753*). In uridine nucleotide depletion disease, one of the earliest beneficial effects of uridine treatment is reduction in the intensity and frequency of seizures that occurs after the first 1-2 weeks.

Important biochemical data on the key role of uridine in regulating the seizure threshold in humans came from the early clinical trials of PALA (N-Phosphonoacetyl-L-Aspartate). PALA is a

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potent inhibitor of *de novo* uridine synthesis via its action on aspartate transcarbamylase (ATC); this agent caused seizures in 11% of patients who received high doses during chemotherapy. ATC is one of three enzymatic activities encoded in a single polypeptide in mammals. This trifunctional protein is called CAD for Carbamoyl phosphate synthetase-Aspartate transcarbamoylase-Dihydroorotase, and catalyzes the first 3 steps in *de novo* pyrimidine synthesis. PALA-induced seizures and brain lesions in rats were prevented by uridine (*Wiley RG. Neurotoxicity of the pyrimidine synthesis inhibitor N-phophsoacetyl-L-aspartate. Ann Neurol 1982;12:175-183.*)

Dr. Yu and Dr. Nyhan at UCSD have used triacetyluridine (PN401) supplied by Pro-Neuron, Inc. (Gaithersburg, MD) in the treatment of children with UNDD and the clinical pentad of seizures, ataxia, language delay, autistic features, and recurrent acute sinusitis over the past 2 years (PNAS 1997;94:11601-11606).

Uridine Prevents Overproduction of Brain Lactic Acid

Isolated perfused brain models showed rapid deterioration of neuronal function and increased lactic acid production. These effects were prevented by adding a perfused liver or uridine was added to the circuit. This showed that the brain protective factor provided by the liver was uridine and cytidine. When uridine and cytidine were added, neuronal function was preserved and lactic acid production was prevented (*Geiger A. Cytidine and uridine requirement of the brain. J Neurochem 1956;1:93-100*).

Anxiolytic and Antidepressant Effects of Uridine

Exogenous uridine was given to patients with bipolar affective and depressive disorders in a Russian population and found to have anxiolytic and antidepressant properties. Uridine binding to GABA, benzodiazepine and imipramine receptors has been demonstrated, but downstream signalling after uridine binding to these receptors has not been characterized (*Karkishchenko NN. Pyrimidine derivatives: their psychotropic properties and the molecular mechanisms of their central action. Farmakol Toksikol 1990;53:67-72 (in Russian, English abstract)*).

Cardioprotective Effects of Uridine

Low-flow myocardial ischemia was studied in isolated, perfused rat hearts. Uridine had no

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effect on n rmally perfused hearts. Ischemia and reperfusion with ut uridine resulted creatine phosphate (CP) and ATP depletion, and l ss of myocardial glycogen. When 50 μM uridine was given during reperfusion, CP and ATP, and glycogen were restored (*Aussedat J. Effect of uridine supply on glycogen resynthesis after ischaemia in the isolated perfused rat heart. Cardiovasc Res 1983;17:145-151*).

Uridine Prevention of Experimental Cerebral Edema

Cerebral edema, increased intracranial pressure (ICP), and neurologic abnormalities consistently follow traumatic, post-ischemic, and cryogenic brain injury. Uridine treatment (inefficiently delivered as UDP) within the first 24 hrs of injury resulted in decreased cerebral edema and reduced ICP toward normal by 58%, and reduced mortality from 21.6% to 0 (*Yoshida S. Effect of uridine 5'-diphosphate on cryogenic brain edema in rabbits. Stroke 1989;20:1694-1699*).

Neurono- and Axonoprotective Effects of Uridine

Uridine supplementation in patients with diabetic neuropathy showed a clear dose response and improved functional status and increased mean nerve conduction velocities (NCV) by 5-10 m/s in patients treated for 6 months (*Gallai V. Effects of uridine in the treatment of diabetic neuropathy: an electrophysiologic study. Acta Neurol Scand 1992;86:3-7*).

Endocrine Effects of Uridine

The role of mitochondrial function in follicular development, ovulation, and the onset of menopause has recently been examined (Hormone Research 1993;39(S1):16-21; Biol Repro 1993;49:730-739; Fertility and Sterility 1995;64:577-583). Mitochondrial DNA deletions in oocytes increase with age and are thought to lead to progressive mitochondrial dysfunction and ovarian senescence associated with menopause. Women with mitochondrial dysfunction who are of reproductive age commonly have menstrual irregularities and premenstrual exacerbation of mitochondrial migraine headaches (unpublished data, R.K. Naviaux, 1998). Women with galactosemia (an inborn deficiency in the enzyme galactose-1-phosphate uridyl transferase) are known to suffer with premature varian failure. The enzyme deficiency in galact semia blocks the

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efficient utilization of uridine in normal galactose and galacto-lipid metabolism. Investigations looking at mitochondria in galactosemic women have not been published. Exogenous supply of uridine to women of reproductive age and mitochondrial disease may complement the functional ovarian deficiency and result in long term improvements in menstrual regularity and reduction in the symptoms associated with premenstrual syndrome.

Uridine Auxotrophy

Cells with defined defects in mitochondrial DNA replication or mitochondrial metabolism are functional auxotrophs for uridine. They are dependent on exogenous uridine for normal growth and metabolism. Without a normal mitochondrial respiratory chain, the rate limiting enzyme in pyrimidine synthesis (DHOD) cannot function, and the cells cannot synthesize enough pyrimidines to survive. Fibroblast cultures established from patients with a number of mitochondrial DNA diseases, such as Kearns-Sayre Syndrome, MELAS, NARP, are frequently best maintained in medium that is supplemented with 20-200 μM uridine and 1 mM sodium pyruvate (*King MP, Attardi G. Science 246:500, 1989*). Without these additives, the rate of cell division slows and many cells die.

Cells established from patients with mitochondrial DNA disease invariably display both intracellular and intercellular heteroplasmy, in which certain cells have a high burden of mutant mitochondrial DNA and other cells have little or none. Growing cells in the absence of uridine results in the death of cells with mutant mitochondrial DNA and survival of cells with little or no mutant mitochondrial DNA. In cell culture, and in the growing embryo, this mechanism of Darwinian cellular selection helps to ensure that rapidly growing cells do not build up large burdens of mutant mitochondrial DNA. However, as cells stop dividing and begin to differentiate in a developing child or aging adult, this selection process can backfire. Postmitotic cells in which mutant mitochondrial DNA is replicated during turnover synthesis, are playing essential functions within an organ. They cannot be deleted without producing functional losses. Under these circumstances, the provision of exogenous uridine and pyruvate may permit cells to survive and to perform their differentiated functions, thus av iding inexorable cellular and functional losses.

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Pyruvate Auxotrophy

Pyruvate has a number of metabolic fates. S me of these fates pose theoretical risks for patients with mitochondrial disease, while others pose significant therapeutic advantages for both cells and patients. In the balance, experience has proven that pyruvate supplementation confers a significant metabolic and growth advantages to cells with impaired mitochondrial function. Human cells lacking mitochondrial DNA, or harboring defective mitochondrial DNA are pyruvate auxotrophs (*King MP, Attardi G. Isolation of human cell lines lacking mitochondrial DNA. Meth Enzymol 264:304-313, 1996*). These cells fail to grow unless 1 mM pyruvate is supplied in the medium. Calcium pyruvate has become a popular nutritional supplement at health food counters, and experience in normal populations has not shown significant toxicity. The current protocol will not supplement with pyruvate, but future studies may be indicated to evaluate the potential synergy between uridine and pyruvate supplementation in mitochondrial disease.

Primary and Secondary Lactic Acidemia

A pyruvate tolerance test may also help to stratify patients with primary lactic acidemia (PLA) and secondary lactic acidemia (SLA). In PLA, lactic acid is produced because of either increased production, decreased elimination, or both, that arise from central defects in mitochondrial metabolism. Primary lactic acidemia may be exacerbated by pyruvate loading. Secondary lactic acidemia is a homeostatic response designed to provide an alternative mobile carbon source, lactate, to cells as a respiratory fuel. I hypothesize that in secondary lactic acidemia, blood lactic acid levels will remain unchanged, or decrease when pyruvate is given. We have preliminary evidence for a related phenomenon. When patients with lactic acidemia were given an alanine load (which is readily converted to pyruvate by transamination catalyzed by alanine transaminase ALT (SGPT)), blood lactic acid did not increase (unpublished data), suggesting the presence of heretofore unrecognized homeostatic controls that defend existing blood levels of lactic acid, even when elevated in mitochondrial disease.

A related phenomenon occurs in Type I glycogenosis (von Gierke disease). These patients normally have elevated blood lactic acid levels. This appears to be a secondary lactic acidemia that

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is defended, i , is compensatory, because when given a glucose challenge, blood lactic acid does not rise, and its transaminated amino acid precursor, alanine, actually falls (Nyhan WL, Ozand PT. In Atlas of metabolic diseases. Chapman and Hall, NY, 1998, p.352)

Clinical Side Effects of Uridine

1. In about 50% of children with pre-existing epilepsy treated with uridine, there appears to be a slight increase in seizure activity in the first 2 weeks of therapy (Naviaux RK and Yu A, unpublished observations). After 2 weeks, seizure activity usually falls to below pre-treatment levels.

2. Mild nausea, vomiting, and/or diarrhea were sometimes seen at the highest doses (0.3 g/kg/d). These symptoms promptly resolved with dose reduction and did not require any additional intervention.

3. Self-limited flares in pre-existing fibrocystic breast disease and shortening of previously irregular menstrual cycles were seen in one 29 year old women with mitochondrial disease. These symptoms spontaneously resolved in 10 weeks, without dose-reduction.

4. Uncomplicated ankle swelling has been seen in some elderly patients (> 60 yo) on PN401. This has been stable and has not required intervention.

5. Transient and uncomplicated reduction in white blood cell count has been seen rarely and has been self-limited.

6. Transient elevation in liver transaminases hase been seen rarely and has been self-limited without need for dose reduction.

No other significant acute or delayed-cumulative toxicity has been encountered in 20 years experience with uridine, and 2 years experience with Triacetyluridine (PN401) in this disease population of suspected or confirmed mitochondrial disease.

Pharmacology of Uridine and Triacetyluridine (PN401)

Uridine has been used in the management of patients with cancer treated with 5-fluorouracil (5-FU) as well as children with defects in pyrimidine metabolism. *Van Groenigen has published pharmacokinetic data for uridine in normal volunteers (JNCI 83:437, 1991).* The bioavailability is

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about 7%. The elimination half-time is 2-4 hrs. The volume of distribution is 0.634 l/kg. Normal plasma uridine levels are 5-13 μM. Therapeutic blood levels of uridine are 50-250 μM. Peak levels were obtained 2-3 hrs after an oral dose. Uracil is the first appearing catabolite. The bioavailability of PN401 is 3-5 times greater than uridine in children and 8-10 times greater in adults. PN401 has also been used in cancer patients with accidental 5-FU overdose at doses of up to 40 g of PN401 per day in adults.

Chronology

1. Morais and his colleagues first showed that animal cells that were deficient in mitochondrial respiration (ρ^0 and ρ^- cells) required supplemental uridine to grow in culture in 1984 (*Gregoire M, Morais R, et al. On auxotrophy for pyrimidines of repsipration-deficient chick embryo cells. Eur. J. Biochem 142:49-55, 1984*). Morais' group also identified the level of the defect to the rate limiting step in *de novo* pyrimidine synthesis, dihydroorotate dehydrogenase (DHOD, EC 1.3.3.1), also called dihydroorotate CoQ-Oxidoreductase (DHO-QO, EC 1.3.99.11) in this pivotal paper.
2. Dr. William Nyhan and Dr. Alice Yu began treating patients with 5' Nucleotidase excess with uridine and with PN401 (triacetyluridine) An abstract describing these patients and their response to PN401 was received by the American Pediatric Society on (*Yu AL, Page T, Fontanesi J, Lawton D, McArthur C, Nyhan W. Pyrimidine responsive syndrome of neurologic dysfunction and susceptibility to infection*; see attached copy). A full description was published in 1997 (*Page T, Yu A, Fontanesi J, Nyhan WL. Developmental disorder associated with increased cellular nucleotidase activity. PNAS 1997;94:11601-11606*).
3. Dr. Naviaux became a fellow in biochemical genetics with Dr. Nyhan and learned of the effectiveness of uridine, and the planned use of PN401 in patients with 5'nucleotidase excess syndrome.
4. Based on clinical similarities with the 5' nucleotidase excess patients (recurrent upper

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respiratory tract infections, seizures, ataxia, and language delay), combined with his knowledge of functional DHOD deficiency in cells with mitochondrial defects, Dr. Naviaux introduced the hypothesis that patients with a variety of mitochondrial diseases are functionally deficient in DHOD and may benefit from therapy with uridine or PN401

5. Dr. Naviaux discussed the concept of functional DHOD deficiency from suspected mitochondrial disease, and the potential benefit of uridine or PN401 therapy with the parents of CMZ (see Murphy-Zink statement.

6. Dr. Naviaux began treating CMZ with uridine

7. Dr. Nyhan and Dr. Yu introduced the concept of mitochondrial disease Inc.,

The discussion of mitochondrial disease was presented in the context of the discovery of polymorphic mitochondrial DNA (mtDNA) mutations found by Dr. Naviaux by Southern blot analysis in 2 of the first 4 patients with 5' nucleotidase excess syndrome. These polymorphic (non-pathogenic) mtDNA mutations were initially thought to be abnormal, but were later discovered to be incidental.

8. Dr. Naviaux was prompted by a medical crisis in a 29 year old female (KL) with mitochondrial disease under his care, to obtain emergency approval from the UCSD Institutional Review Board (IRB) and the Food and Drug Administration (FDA) for an emergency IND for PN401 to treat KL and CMZ. In order to obtain the drug, Dr. Naviaux approached Pro-Neuron, Inc., Gaithersburg, MD.

9. Dr. Naviaux submitted a proposal for a study IND to the FDA for a formal, investigator-initiated, clinical trial of PN401 in the treatment of mitochondrial disease in see

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ABSTRACT

The present invention provides methods for treating mitochondrial-related disorders, wherein the disorder is characterized by a uridine deficiency, comprising administering to a subject in need of such treatment, a therapeutically effective amount of triacetyluridine (PN401), thereby relieving or ameliorating at least one symptom of the disorder. Over 40 exemplary mitochondrial-related disorders are provided herein, as are descriptions of many of the symptoms associated with mitochondrial-related disorders.